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14. ABSTRACT

Over the last reporting period we have (Aim 1) developed an interleaved Na^+/H^+ MR spectroscopic sequence, interleaved gradient echo (GRE) imaging sequence, and completed an initial prototype of our initially proposed interleaved pH, O_2, and sodium-weighted sequence. We have enrolled 6 pre-operative patients (Aim 2) and obtained image-guided biopsies of areas thought to have high acidity, hypoxia, and sodium concentration with the goal of next correlating with tissue biology including NHE1 expression on IHC and RNA sequencing, as well as correlating with bioenergetics once we reach 50% recruitment. Lastly (Aim 3), we have obtained pre- and post-immunotherapy pH, O_2, and sodium-weighted MR images in 5 recurrent glioblastoma patients with the goal of next correlating with changes in patient outcome and tissue biology. Initial results suggest a positive correlation between change in imaging measures of rNHE and change in tumor volume after immunotherapy, as well as a correlation between a sodium concentration and diffusion MR measurements, pH imaging measurements, and absolute tumor volume.

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1. Introduction

Glycolysis is often enhanced in cancers, *even in the presence of abundant oxygen* (i.e. the Warburg effect). This form of *aerobic glycolysis* results in a significant decrease in extracellular pH due to increased concentration of lactic acid and other factors. Maintaining pH regulation during excessive metabolism requires active transport of protons out of tumor cells. In brain cancer, this is done not only through lactate transport, but also through activity of sodium (Na^+)-proton (H^+) exchanger isoform-1 (NHE1). NHE1 is coupled with pH homeostasis in glioma cells and plays a role in in the progression of malignant gliomas as well as treatment resistance to both chemotherapies and immunotherapies, presumably because extracellular acidity has been linked to elevated mutagenesis and chromosomal rearrangements, elevated p53 and p21 expression, increased tumor invasion, formation of cancer stem cells, decreased immune function, and increased pro-angiogenic signaling. We hypothesize images of tumor acidity, oxygen consumption, and salinity (sodium concentration) can be performed in clinically realistic time frames through interleaving fast multinuclear Na^+/H^+ image acquisition. Such data will be useful for studying NHE1 expression and function in human brain tumors, including predicting immunotherapy treatment response. To accomplish this goal, we propose (Aim 1) constructing and testing a novel multinuclear Na^+-H^+ metabolic MRI sequence with sensitivity to Na^+ concentration, tissue pH, and O_2 utilization. Then (Aim 2) we will correlate Na^+-, pH-, and O_2-weighted MR image measurements with NHE1 IHC, bioenergetics, and gene expression using stereotactic image-guided biopsies from human brain tumors. Lastly, (Aim 3) we will quantify changes in Na^+-, pH-, and O_2-weighted MR images after neoadjuvant anti-PD-1 immunotherapy in recurrent GBM and explore associated changes in tumor biology.

2. Keywords

Glioblastoma; metabolic imaging; multinuclear MRI; sodium MRI; brain tumor; immunotherapy

3. Accomplishments

Aim 1: Construct and test a novel multinuclear Na+-H+ metabolic MRI sequence with sensitivity to Na+ concentration, pH, and O_2.

Major Task 0: IRB and HRPO Approval
Major Goals: IRB and HRPO Approval for Aim 1
Accomplishments: Within the first 3 months we accomplished this key objective and established IRB and HRPO approval. This task is complete.

Major Task 1: Sequence Programming
Major Goals: Implement a prototype Na+/H+-CEST-SAGE-EPI sequence.
Accomplishments: During the last 12 months we have been working diligently with Siemens and collaborators to overcome software and firmware challenges associated with switching between multiple nuclei within the same pulse sequence. This seemingly simple task is critical to accomplish the goal of this Aim, yet Siemens engineers have yet to fully figure this out. Siemens has admitted that (1) this has never been done before and (2) they do not know how to solve this issue even on their own systems. Dr. Bydder and Dr. Yao, our project scientist and postdoctoral fellow last year, have since left UCLA and Dr. Chencai Wang, another project scientist with an expertise in MRI pulse sequence design, has been working with Dr. Xiaodong Zhong, a Siemens engineer, to overcome these issues. In response to this need, however, Siemens has dedicated significant resources to help us figure out how to accomplish this goal.

Despite this critical setback, we have made several important accomplishments over the last 3 months and are pleased to report we are close to fully completing this task. First, we have successfully acquired an interleaved, multinuclear spectroscopic free induction decay (FID) signal from both sodium and proton nuclei (or any X-nuclei, including ^{31}P) (**Fig. 1A-C**). Next, we extended this basic spectroscopic sequence to include an interleaved multinuclear fast low angle shot gradient echo *imaging* sequence (^{1}H-FLASH/^{23}Na-FLASH) (**Fig. 1D-E**), and demonstrated feasibility using a simple saltwater phantom (**Fig. 1F-I**). Expanding on this exciting milestone, we recently coded and successfully compiled a *single echo* ^{1}H-CEST-EPI/^{23}Na-FLASH sequence (**Fig. 1J-K**), a critical step allowing pH and sodium-weighted image information simultaneously. Despite compiling, this sequence is currently causing errors when testing on phantoms and remains the focus of our collaborative efforts with Siemens. After we overcome this issue, the last and final step will be coding, compiling, and testing the *multi-echo* ^{1}H-CEST-*SAGE*-EPI/^{23}Na-FLASH sequence, which will provide the desired pH, O_2, and sodium-weighted imaging information. We are optimistic and believe we will have this accomplished by the end of the calendar year.

It is important to note that we have also had significant *technical issues with the multinuclear head coil* during the past year and have had to send the coil back to RAPID for testing and repair in Germany (3/21/22 – 7/8/22). Due to COVID-19 and transportation delays, this process took longer than expected. These technical issues caused severe artifacts (summarized in **Fig. 3** and described under **Aim 3**) and limited the productivity of **Aim 1** as limited testing could be performed when the coil was being repaired (see **Fig. 6** for timeline).

Major Task 2: Phantom Testing
Major Goals: Repeated testing on a custom phantom to ensure stability and accuracy of the new MRI pulse sequence.
Accomplishments: We have constructed a saltwater phantom for use in the current project as outlined in **Fig. 1E-F**. However, this major task is dependent on successful completion of Major Task 1, which is still under development as outlined above. Once a stable sequence is completed, we will perform repeated testing to ensure stability and accuracy of the combined sequence.

Major Task 3: Human Testing
Major Goals: Test the stability and repeatability of the MRI pulse sequence in 20 healthy volunteers.
Accomplishments: We have an IRB in place for testing healthy human volunteers, but this task is dependent on Major Task 1, which is still under development as outlined above. Once a stable sequence is completed, we will test the stability and repeatability of the sequence in 20 healthy volunteers.

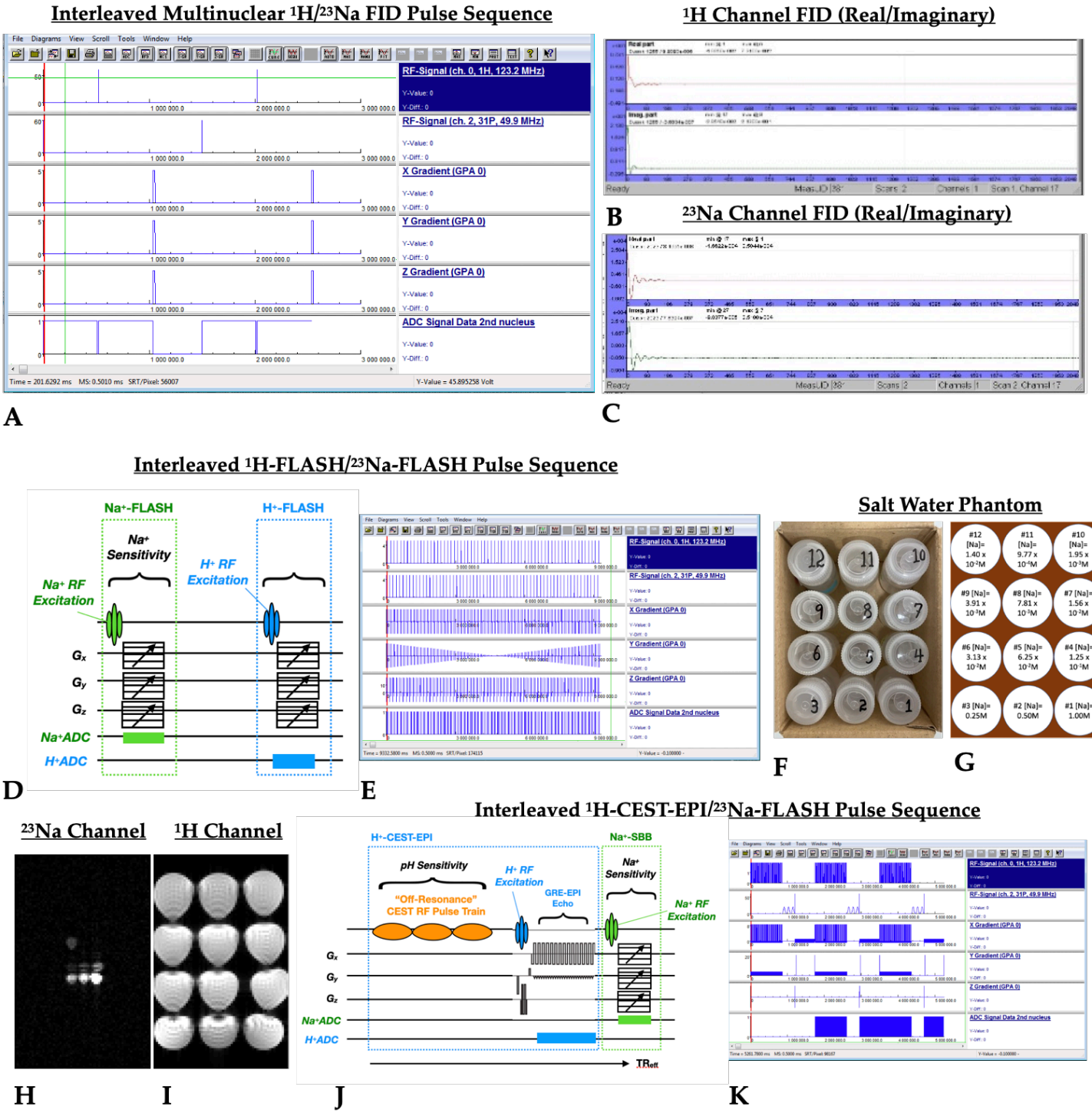

Fig. 1. A) Interleaved multinuclear $^{1}H/^{23}Na$ spectroscopic FID pulse sequence diagram in Siemens IDEA software. **B)** Resulting proton (^{1}H) and **B)** sodium (^{23}Na) FID including both real (top) and imaginary (bottom) quadrature channels. **D)** Interleaved, multinuclear gradient echo *imaging* (FLASH) pulse sequence diagram and **E)** this diagram implemented in Siemens IDEA software environment. **F-G)** Constructed saltwater phantom with different concentrations of sodium. **H)** Simultaneous sodium and **I)** proton FLASH images of the saltwater phantom. **J)** Interleaved *single echo* ^{1}H-CEST-EPI/^{23}Na-FLASH pulse sequence diagram and **K)** this diagram implemented in Siemens IDEA software environment to run on the 3T Prisma scanner.

Aim 2: Correlate Na^+-, pH-, and O_2-weighted MR image measurements with IHC, bioenergetics, and gene expression using stereotactic image-guided biopsies from human brain tumors.

Major Task 0: IRB and HRPO Approval
Major Goals: IRB Approval for Aim 2.
Accomplishments: Within the first 3 months we accomplished this key objective and established IRB and HRPO approval. This task is complete.

Major Task 1: Surgical Patient Recruitment
Major Goals: Start recruitment of newly diagnosed or recurrent gliomas.
Accomplishments: To date, we have recruited **6 patients** with new or recurrent gliomas to the study, which is lower than our expected recruitment of 1-2 patients per month for the first 18 months. This low recruitment was due in part to (1) difficulties recruiting patients for extra clinical time due to COVID-19 concerns; (2) difficulties scheduling patients on the dedicated research scanner on campus; (3) technical issues associated with the multinuclear head coil resulting in significant image artifacts (summarized in **Fig. 3)**; and (4) periodic scanner downtime due to unrelated technical issues with the MR scanner itself (8/14/22 - present). In the past few months, we have actively increased recruitment and have another 4 or so more patients actively enrolled in the next few weeks once the gradient coil on the scanner is replaced. (see **Fig. 6** for timeline of patient enrollment and scanner downtime)

Major Task 2: Na+/H+ Pre-Surgical MRI
Major Goals: MRI exam including exploratory Na+/H+ sequences, the international standardized brain tumor imaging protocol (BTIP), diffusion and perfusion imaging.
Accomplishments: We have successfully acquired multinuclear imaging data in all 6 patients we have enrolled (**Fig. 2**). The exam is about 90 minutes and covers the standard anatomic sequences within BTIP along with sodium MRI acquired at the very end, after contrast administration. We will continue to acquire pre-surgical multinuclear MRI scans in the remaining 14 patients over the next year.

Major Task 3: Surgery & Correlation with Tissue
Major Goals: Surgery and correlation with tissue.
Accomplishments: We have successfully collected more than 12 biopsy samples in the 6 enrolled patients (Subtask 1). All tissue is currently banked and bioenergetics/seahorse (Subtask 2), NHE1 RNA seq (Subtask 3), and NHE1 IHC staining (Subtask 4) will be performed as a single batch as soon as 10 patients are enrolled (50% of the total enrollment for this aim).

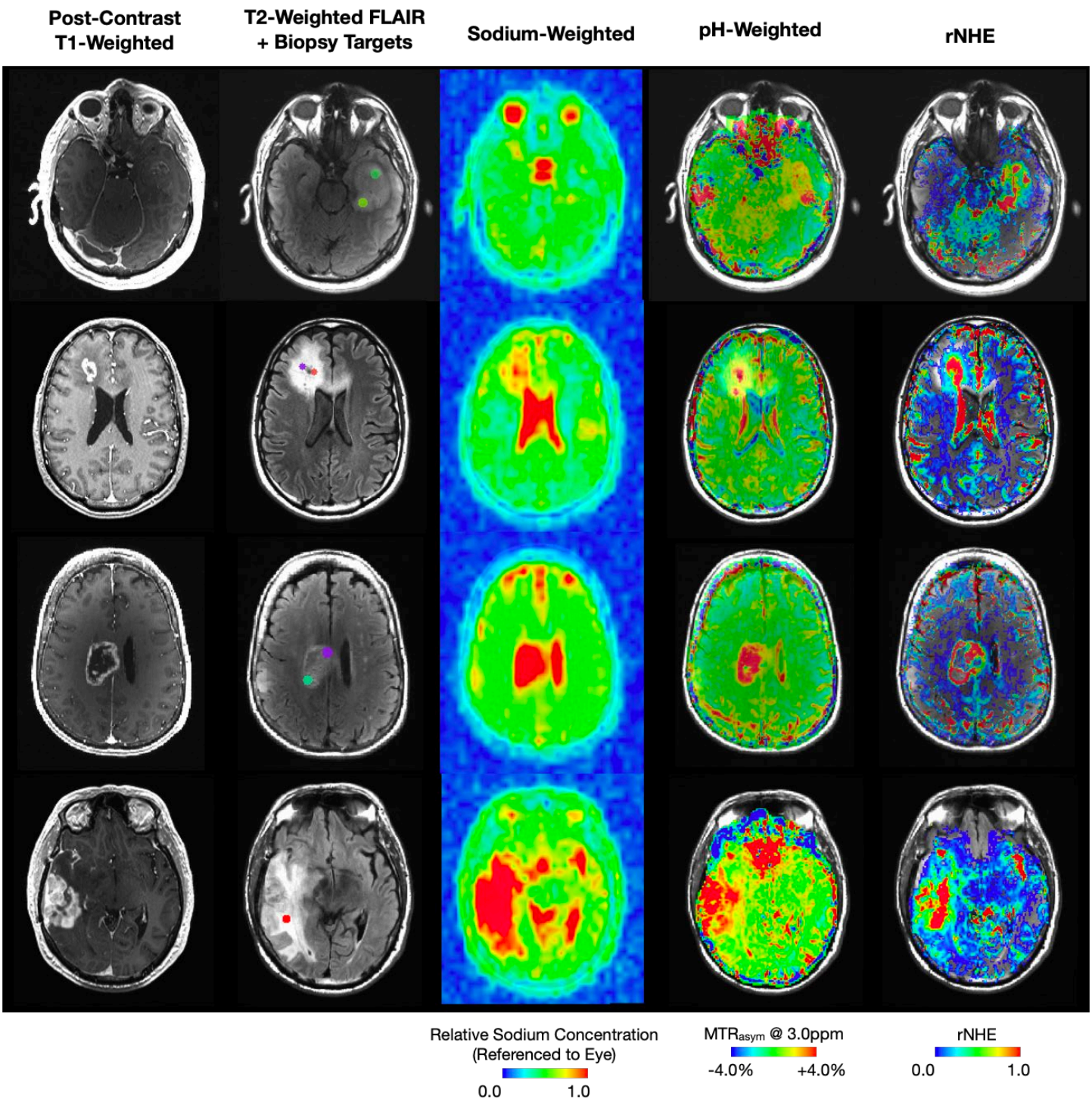

Fig. 2. Multinuclear surgical patients enrolled for Aim 2. (Left to right) Post-contrast T1-weighted images, T2-weighted FLAIR images, sodium-weighted images, pH-weighted amine CEST-SAGE-EPI images, and relative sodium-hydrogen exchanger (rNHE) images based on a combination of sodium-, pH-, O_2-, diffusion-, and perfusion-weighted images. On FLAIR images (2^{nd} column from left), colored spheres show biopsy targets.

Aim 3: Quantify changes in Na+-, pH-, and O2-weighted MR images after neoadjuvant anti-PD-1 immunotherapy in recurrent GBM and explore associated changes in tumor biology.

Major Task 0: IRB and HRPO Approval

Major Goals: IRB Approval for Aim 3.

Accomplishments: Within the first 3 months we accomplished this key objective and established IRB and HRPO approval. This task is complete.

Major Task 1: Recurrent Patient Recruitment

Major Goals: Start recruitment of recurrent glioblastoma patients being treated anti-PD-1 immunotherapy.

Accomplishments: We have successfully recruited **5 patients** with recurrent glioblastoma undergoing anti-PD-1 immunotherapy, including 1 screen fail (patient did not receive treatment, so they did not get a 2nd MRI exam). Recruitment has been slow due to technical issues with the multinuclear coil, as shown in **Fig. 3.** In particular, the last few patients we scanned (see **Fig. 6** for timeline – patients marked with a * had some artifacts) had a series of very specific artifacts that are likely caused by radiofrequency contamination and/or faulty connections. Importantly, despite these technical issues that have limited our recruitment time period, we have enrolled a substantial number of patients and performed numerous exams during the periods the scanner and equipment was available (**Fig. 6**).

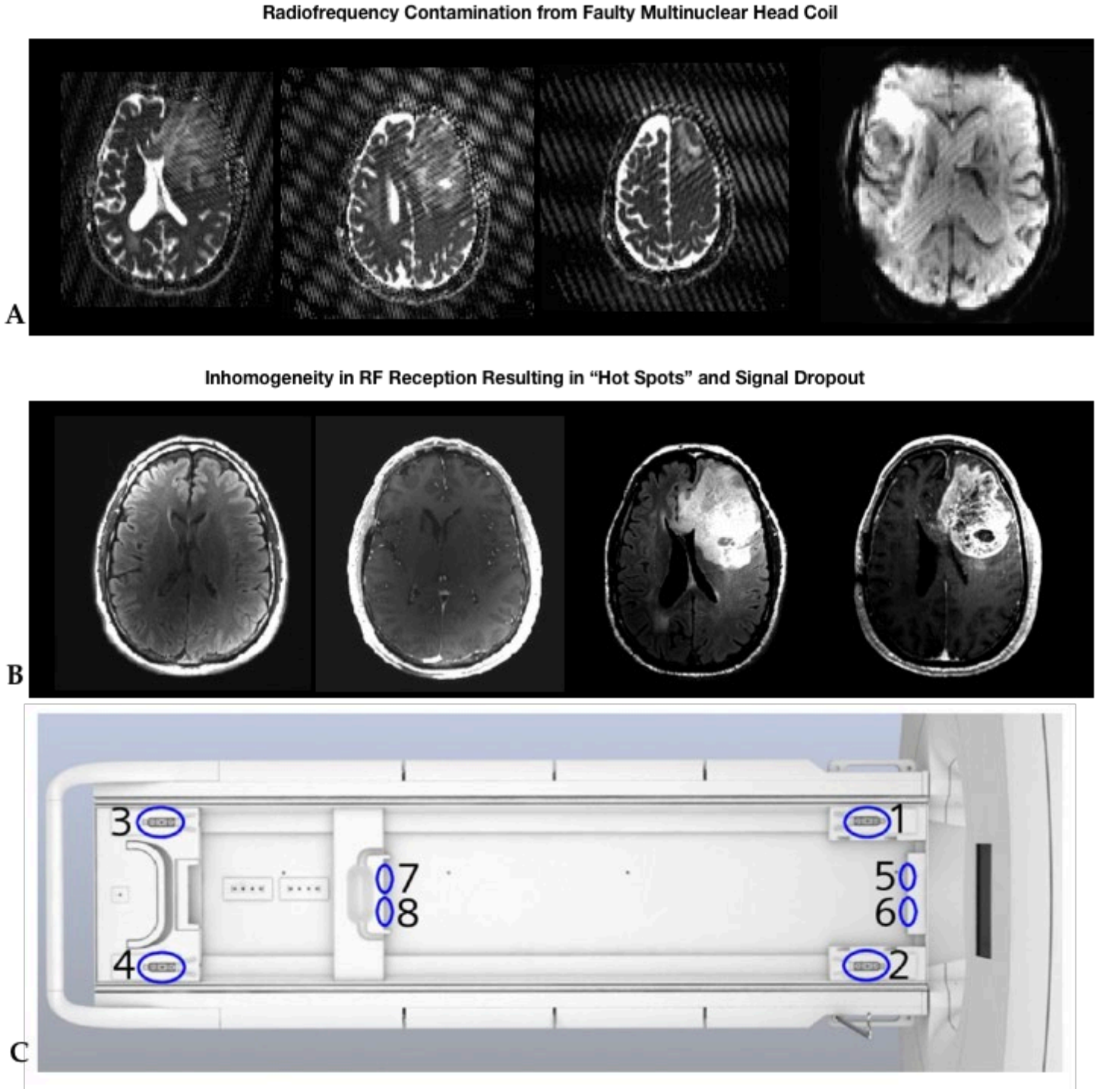

Fig. 3. Representative artifacts observed as a result of technical issues with the multinuclear head coil. A) External radiofrequency (RF) contamination of the MR signal from a specific frequency (spike in *k*-space) or band of frequencies resulting in striping of the images, particular in EPI-based images including CEST-EPI, diffusion, and perfusion MRI. **B)** Inhomogeneity in the received MR signal from faulty $^{+}$H channels resulting in "hot spots" and signal dropout in both FLAIR and T1-weighted anatomic images. As a result of these artifacts acquired in study patients, we sent the coil to RAPID Biomedical for repairs. This process of sending the coil, testing by RAPID, and sending the coil back postponed recruitment of patients into Aims 2 and 3. **C)** Coil connections on the scanner bed. The MRI bore is to the right on this figure. Currently, the sodium channel plugs into (2) and the proton channels plug into (1) and (3). We noticed issues with the proton coil connection in (3), where the pins may be exposed to RF energy during the exam resulting in RF artifacts as illustrated in **A**. Additionally, if there is tension on the wires connecting from the head coil (placed near (5-6)) and the connection in (3), this can cause signal drop out as illustrated in **B.**

Major Task 2: Pre/Post Treatment Scanning

Major Goals: Major goals for this task include acquiring a pre-treatment multinuclear MRI exam (Subtask 1), treatment with anti-PDL1 immunotherapy (Keytruda) (Subtask 2), and acquiring a post-treatment multinuclear MRI exam (Subtask 3) prior to surgery.

Achievements: We have successfully acquired pre-treatment multinuclear MRI exams in **5 patients** enrolled in Aim 3, but only treated and obtained a post-treatment multinuclear MRI exam in 4 of the 5 patients initially enrolled (**Fig. 4**). One of the patients had a screen fail for the therapeutic trial and was not eligible to receive anti-PD1 treatment, so we did not acquire the follow-up multinuclear MRI exam. Similar to Aim 2, this low recruitment was due in part to (1) difficulties recruiting patients for extra clinical time due to COVID-19 concerns; (2) difficulties scheduling patients on the dedicated research scanner on campus; (3) technical issues associated with the multinuclear head coil resulting in significant image artifacts (summarized in **Fig. 3)**; and (4) periodic scanner downtime due to unrelated technical issues with the MR scanner itself (8/14/22-present). In addition to these concerns, there has been a pause on the specific immunotherapy trial we have been evaluating using the multinuclear sequence, so recruitment has been slower than expected. However, a new immunotherapy trial (i.e. nivolumab plus ipilimumab)

Despite these setbacks, the preliminary data is quite interesting (**Fig. 4-5**). In the 3 patients with reliable, artifact-free data (NA001-003), some enhancing lesions exhibited an increase while some exhibited a decrease in relative sodium-proton exchanger (rNHE) index after immunotherapy (**Fig. 5A**), even though the volume of the enhancing lesion increased in all cases. While data is limited, it does appear that the change in rNHE is positively associated with the change in contrast enhancing volume as theorized (**Fig. 5B;** R^2*=0.9944, P=0.0475*).

Major Task 3: Correlation with Tissue

Major Goals: Surgery and correlation with tissue.

Accomplishments: We have successfully collected tissue from patients enrolled in this aim. All tissue is currently banked, and analysis is ongoing in collaboration with Dr. Rob Prins. Results will be evaluated as soon as 10 patients are enrolled (50% of the total enrollment for this aim).

Major Task 4: Correlation with Outcomes

Major Goals: Correlate rNHE response with progression-free (PFS) and overall survival (OS).

Accomplishments: To date, none of the patients enrolled in Aim 3 have died, but 3 of the 5 have exhibited radiographic progression following surgical resection after immunotherapy. No association was observed between rNHE measurements and PFS (*R^2=0.1597, P=0.7983, plots not shown*).

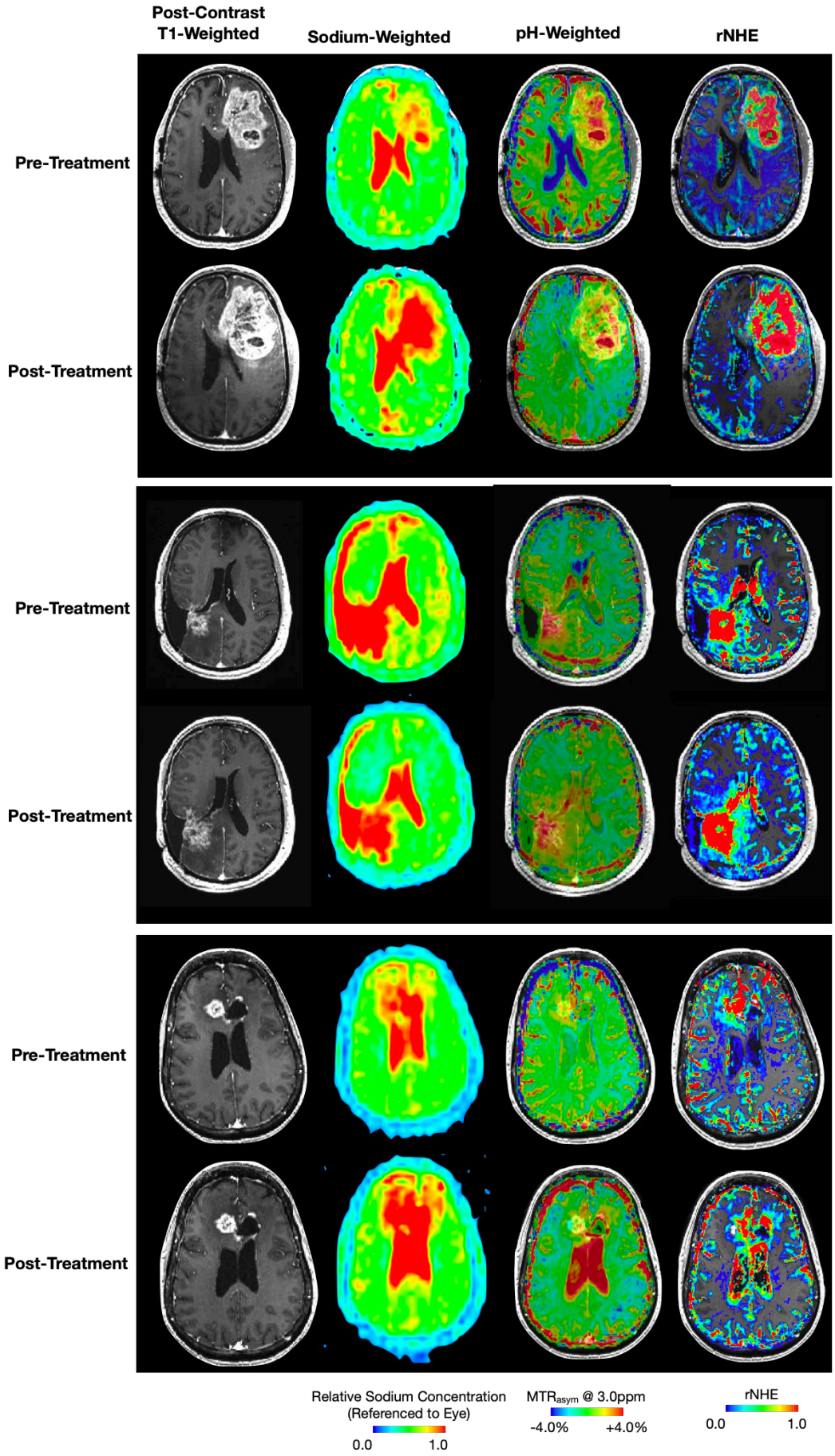

Fig. 4. Post-contrast T1-weighted, sodium-weighted, pH-weighted, and relative sodium-proton exchanger (rNHE) index changes pre- and post-immunotherapy (immune checkpoint inhibition) in three patients with recurrent IDH wild type glioblastoma. Results from this limited patient sample demonstrate feasibility of performing the proposed complex multinuclear MRI exam, despite lack of therapeutic efficacy using this type of immunotherapy.

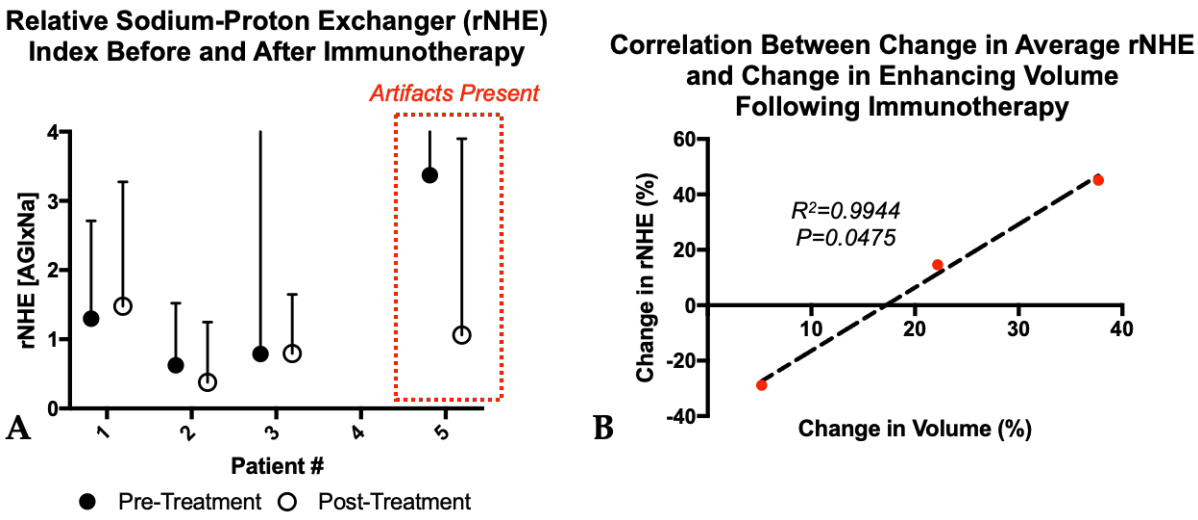

Fig. 5. Change in average rNHE before and after check point inhibition in IDH wild type recurrent glioblastoma. A) Pre- and post-treatment average rNHE measurements (error bars = SD within enhancing tumor). Note patient #4 failed screening and did not receive treatment (or a post-treatment MR exam) and patient #5 had significant artifacts on the pre-treatment scan and was excluded from subsequent analyses. (The coil was sent for repairs shortly after scanning patient #5). **B)** Correlation between change in average rNHE and change in contrast enhancing tumor volume after immunotherapy. While preliminary, results suggest a decrease in rNHE is associated with an increase in tumor volume, consistent with our initial hypotheses.

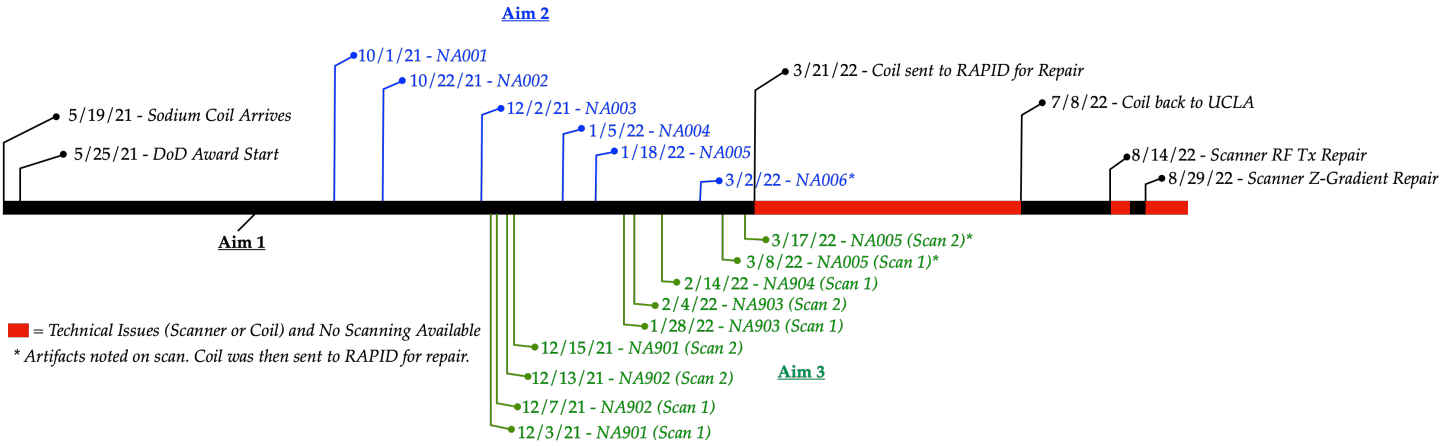

Fig. 6. Study timeline from the start of the award to present date. The first 5-6 months after the start of the award was focused on pulse sequence development and testing, as well as getting protocols in place to start human scanning (Aim 1). The first brain tumor patients started enrollment and scanning on 10/1/21 (for Aim 2) and patients were scanned for Aims 2 and 3 until the coil was sent to RAPID in Germany on 3/21/22 for repairs. From 10/1/21 to 3/17/22 (~5.5 months), a total of 11 patients and 15 exams were performed (average of ~2.7 exams per month). From 3/21/22 to 7/8/22 the coil was undergoing repair and testing, then was sent back to UCLA. From 7/8/22 to around 8/1/22 we underwent QC/QA testing of the coil to continue to troubleshoot issues associated with artifacts. On 8/14/22, the 3T Prisma scanner with the multinuclear package had a radiofrequency transmitter (RF Tx) channel fail. The scanner was back online for a few days until 8/29/22 where the z-gradient on the scanner went down for repair. As of today (9/15/22), the scanner is still down and undergoing repairs.

Additional Findings

One of our secondary goals for this project was to determine the association between these multiparametric and multinuclear measurements to see of some of the information is redundant. For example, the apparent diffusion coefficient (ADC) is a measure of translational molecular motion of water molecules, while measures of proton T2 is associated with *rotational* molecular motion of water molecules. Thus, both ADC and T2 measurements are thought to reflect *extracellular water mobility* or concentration, where water is less bound to surface structures and more freely diffusing. Since extracellular fluid also contains a high concentration of sodium ions, we theorized that ADC (and T2) would be highly correlated with sodium concentration. Consistent with this hypothesis, average (normalized) sodium concentration was significantly correlated with water proton ADC in both areas of contrast enhancement (**Fig. 7A;** R^2*=0.87, P<0.0001*) and T2 FLAIR hyperintense regions (**Fig. 7B;** R^2*=0.77, P<0.01*). Interestingly, areas of macroscopic necrosis (i.e. centrally localized hypointense regions on post-contrast T1-weighted images) did not show this same association (**Fig. 7C;** R^2*=0.19, P=0.24*). Additionally, we noticed there was no significant correlation between pH-weighted and sodium-weighted image contrast in contrast enhancing tumor (**Fig. 7D;** R^2*=0.30, P=0.10*), suggesting they may be reflecting differing aspects of the tumor microenvironment. However, areas of necrosis which are known to be highly acidic do seem to be significantly associated with increasing sodium concentration (**Fig. 7E;** R^2*=0.54, P<0.05*). Lastly, we noted a strong and statistically significant association between average rNHE in enhancing tumor, calculated using a combination of pH-, oxygen-, sodium-, perfusion- and diffusion-weighted image, and the absolute volume of contrast enhancing tumor (**Fig. 7F;** R^2*=0.8479, P<0.0001*). This suggests that tumors may express higher levels of the sodium-proton exchanger and/or have greater degrees of deeply hypoxic/acidic tissue as tumors get larger. This is also consistent with our initial hypotheses, suggesting NHE expression is highest in deeply hypoxic regions that occur when tumors outgrow their nutrient and oxygen supply.

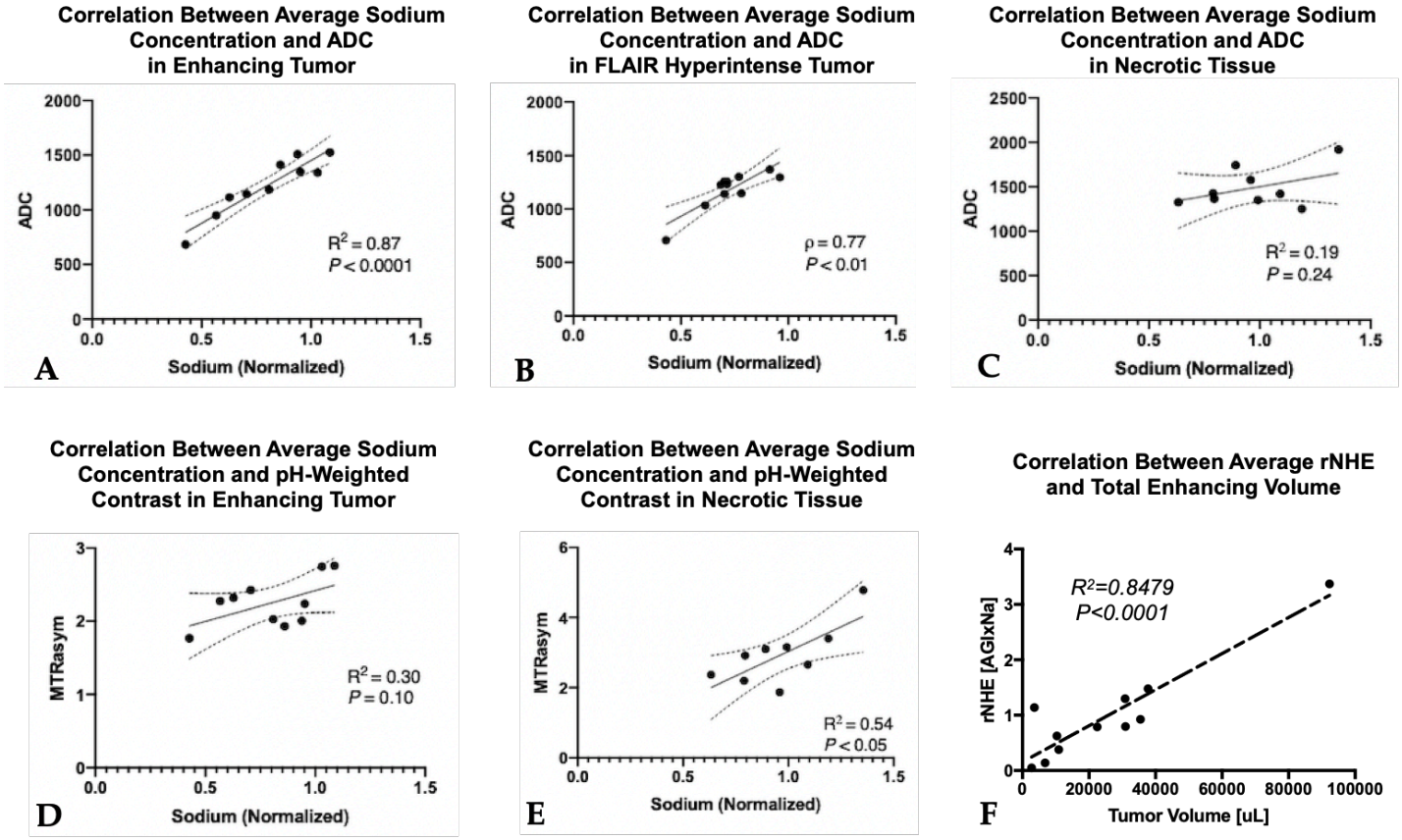

Fig. 7. Correlation between proton and sodium MRI measurements in human gliomas. A) Correlation between water proton apparent diffusion coefficient (ADC) and sodium concentration (normalized to the eye) in contrast enhancing tumor, **B)** T2 FLAIR hyperintense tumor regions, and **C)** areas of macroscopic necrosis (in a subset of gliomas with necrosis). **D)** Correlation between sodium concentration and pH-weighted image contrast (MTR_{asym} at 3ppm, in %) in contrast enhancing tumor and **E)** macroscopic necrotic tissue. **F)** Correlation between the proposed composite, multinuclear rNHE index and absolute volume of contrast enhancing tumor.

Opportunities for Training and Professional Development

This project has led to a few training and professional development opportunities. Nicholas Cho, an MD/PhD graduate student in my laboratory, has been spearheading this project. This project has allowed him training in image acquisition and analyses, and he has learned numerous skills related to image-guided biopsy from Aim 2. Nicholas has attended a number of surgeries and has obtained precise targets in the O.R. as neurosurgeons take out the tissue for subsequent analyses. Additionally, Nicholas has submitted an abstract and has had a poster accepted relating to the data in **Fig. 7** to the *Society of Neuro Oncology (SNO) Annual Meeting* this fall in Tampa.

Dissemination of Results

Some of the data in **Fig. 4** was presented at the Society of Neuro Oncology (SNO) and American Society of Clinical Oncology (ASCO) Meeting on CNS Clinical Trials in Toronto August 11, 2022. At this meeting, we described our approach as a way of understanding changes in the microenvironment after immunotherapy and as an example of imaging technology on the horizon.

An abstract related to the data in **Fig. 7** has also been accepted as a poster presentation at the 2022 SNO Annual Meeting.

All other data shown above has not been disseminated yet, but will be drafted into a publication as soon as more patient data has been acquired.

Plan to Accomplish Remaining Goals During Next Reporting Period

We are very close to completion of the complex multinuclear MRI pulse sequence outlined for **Aim 1.** We are actively hiring both a postdoctoral fellow as well as a staff scientist focused on X-nuclei imaging including this project. Dr. Chencai Wang has been in contact with Dr. Alfredo Lopez from the NMR Laboratory in the Neuromuscular Investigation Center at the Institute of Myology in Paris, France, and he has agreed to come to UCLA for a year sabbatical to help with multinuclear projects including the pulse sequence proposed for **Aim 1**. If the head coil and the MR system itself remain functional, which has been a moderate issue with this project, we anticipate this aim will be completed in the next 6 months.

We are prioritizing and rapidly increasing patient enrollment for **Aims 2-3** over the next reporting period to reach our proposed target enrollment goals. Due to technical issues with the coil and MR scanner itself (**Fig. 6**, red regions on the timeline), we were not able to continue enrollment at our normal pace (~2.7 scans per month). Therefore, we will double our efforts when the scanner is back up and aim for a significantly higher enrollment rate.

4. Impact

Impact on the Development of Principle Disciplines of the Project

As mentioned in Section 3, we have disseminated our initial findings at recent neuro oncology and brain tumor clinical trial scientific meetings. At these meetings we described how our approaches to understanding the interaction of sodium concentration, acidity, and hypoxia within the microenvironment may help in prediction of which patients may respond to immunotherapies, and whether changes in tumor size that may be due to inflammation alter the tumor microenvironment. Our preliminary data initiated discussion regarding how this tool, and novel multinuclear, composite biomarkers could be used to better understand how characteristics of the tumor microenvironment may impact therapeutic mechanisms of action and ultimately treatment efficacy.

Impact on Other Disciplines

Some of the technology and approaches initially conceptualized in our application has had an impact on other disciplines, particularly in neuroscience. Our collaborator Alfredo Lopez from France, who helped us with critical issues associated with acquiring both proton and sodium nuclei in the same sequence that Siemens was struggling to figure out, has recently published a review paper a new emerging field of interleaved and simultaneous multinuclear MRI (*Lopez Kolkovsky AL, Carlier PG, Marty B, Meyerspeer M. Interleaved and simultaneous multi-nuclear magnetic resonance in vivo. Review of principles, applications and potential. NMR Biomed. 2022 Oct;35(10):e4735. doi: 10.1002/nbm.4735. Epub 2022 Apr 27. PMID: 35352440.*). Through our collaborations, he has also published a few articles looking at dynamic interleaved imaging in the calf muscle (Lopez Kolkovsky AL, Marty B, Giacomini E, Meyerspeer M, Carlier PG. Repeatability of multinuclear interleaved acquisitions with nuclear Overhauser enhancement effect in dynamic experiments in the calf muscle at 3T. Magn Reson Med. 2021 Jul;86(1):115-130. doi: 10.1002/mrm.28684. Epub 2021 Feb 9. PMID: 33565187.) as well as using proton images for motion correction of sodium images (*Wilferth T, Müller M, Gast LV, Ruck L, Meyerspeer M, Lopez Kolkovsky AL, Uder M, Dörfler A, Nagel AM. Motion-corrected 23 Na MRI of the human brain using interleaved 1 H 3D navigator images. Magn Reson Med. 2022 Jul;88(1):309-321. doi: 10.1002/mrm.29221. Epub 2022 Apr 4. PMID: 35373857.*)

Additionally, we have also been collaborating with Siemens engineers about our interleaved multinuclear approach, including but not limited to the sequences in **Fig. 1.** They are very interested in this approach in order to speed up acquisition on their ultra-high field (UHF) 7T MR scanners. We anticipate this approach will have a significant impact on the UHF and multinuclear MRI field for some time to come.

Impact on Technology Transfer

We are working in collaboration with Siemens on this project and therefore anticipate our technology to be useful on their multinuclear MRI systems including UHF systems.

Impact on Society Beyond Science and Technology

Nothing to Report

5. Changes/Problems

Changes in Approach and Reasons for Change

We have not changed our approach during the reporting period.

Actual or Anticipated Problems or Delays and Actions or Plans to Resolve Them

As mentioned in Section 3, we have had 2 major problems with the current project: (1) issues associated with technically acquiring two different nuclei in the same pulse sequence, a critical step in building a multinuclear MRI pulse sequence; and (2) technical issues with the multinuclear MRI head coil and MR system itself, which stopped both sequence development (Aim 1) as well as patient recruitment (Aims 2-3).

To overcome our first issue associated with technically being able to acquire two nuclei in the same sequence, we first employed the help of Siemens engineers as we are trying to implement this on their system and multinuclear MR spectroscopic sequences (similar to the sequence shown in **Fig. 1A**) have been used since the late 1980s. Unfortunately, no one at Siemens was able to help us, as most of the multinuclear knowledge within Siemens is gone or has moved to UHF 7T MR systems that run under a completely different system architecture. Since Siemens was not helpful, we reached out to Armin Nagel in Erlangen, Germany, who is thought to be the world expert in sodium MRI. After some fruitful discussions, he suggested we reach out to Dr. Alfredo Lopez in France who has been working recently on this issue and was also frustrated with the lack of support from Siemens engineers. Dr. Chencai Wang from my team has been having weekly meetings with Dr. Lopez, and Dr. Lopez was instrumental in getting to where we are with the multinuclear interleaved sequences shown in **Fig. 1**. We feel through his collaboration we have made significant progress in developing this very complex imaging technique and we should have a working prototype in a few months.

With respect to technical issues associated with the multinuclear MRI head coil and MR system itself, we believe we have fixed the technical issues associated with the head coil after sending to RAPID for repairs over the summer. The MR system is currently down and a gradient is being replaced, which we anticipate to be finished by the end of the month (Sept. 2022). We have numerous patients scheduled for both Aim 2 and Aim 3 starting in October and we are optimistic we will be able to increase enrollment and finish Aims 2-3 by the next reporting cycle.

Changes that had a Significant Impact on Expenditures

Nothing to report.

Significant Changes in Use or Care of Human Subjects

Nothing to report.

The IRB for Aim 1 (IRB#21-000234) is currently approved until 1/23/2023.
The IRB for Aims 2-3 (IRB#21-000514) is currently approved until 3/23/2023

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

Individuals that have worked on the project

Name:	Benjamin M. Ellingson, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (ORCID ID):	0000-0002-2764-6640
Nearest Person Month Worked:	2.4
Contribution to the Project:	Dr. Ellingson provides oversight and expertise for all aspects of the current study. He is involved in pulse sequence programming tasks, enrolling and scanning patients, performing image analysis, and identifying biopsy targets.
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant; NIH/NCI R01 grant; NIH/NINDS R01 grant

Name:	Jingwen Yao, Ph.D.
Project Role:	Graduate Student
Researcher Identifier (ORCID ID):	0000-0002-8828-1841
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Yao was involved in early pulse sequence programming and image analysis tasks (Aim 1) before she left the University for fellowship in July 2021.
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant

Name:	Mark Bydder, Ph.D.
Project Role:	Staff Scientist
Researcher Identifier (ORCID ID):	0000-0001-9210-0225
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Bydder was involved in early pulse sequence programming tasks (Aim 1) before he left the University in late 2021.
Funding Support:	UCLA Department of Radiology support

Name:	Chencai Wang, Ph.D.
Project Role:	Staff Scientist/Programmer
Researcher Identifier (ORCID ID):	0000-0003-0501-3073
Nearest Person Month Worked:	2.4
Contribution to the Project:	Dr. Wang has performed work related to designing and implementing the multinuclear MRI sequence (Aim 1).
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant; NIH/NCI R01

Name:	Nicholas Cho, B.S.
Project Role:	Graduate Student
Researcher Identifier (ORCID ID):	0000-0002-8686-0575
Nearest Person Month Worked:	2.4
Contribution to the Project:	Mr. Cho is involved in image acquisition, post-processing, and analysis for Aims 2-3.
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant; UCLA NIH T32 Medical Scientist Training Program

Name:	Francesco Sanvito, M.D.
Project Role:	Visiting Medical Scholar
Researcher Identifier (ORCID ID):	0000-0002-9152-9684
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Sanvito has assisted in image acquisition, post-processing, and analysis for Aims 2-3.
Funding Support:	UCLA Department of Radiology Support

Name:	Catalina Raymond, M.S.
Project Role:	Lead Programmer
Researcher Identifier (ORCID ID):	0000-0003-2757-439X
Nearest Person Month Worked:	1.0
Contribution to the Project:	Ms. Raymond is responsible for data processing, QC/QA, storage, and analysis for all aspects of the project.
Funding Support:	UCLA Department of Radiology Support

Name:	David Nathanson, Ph.D.
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-4919-9159
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Nathanson is responsible for tissue processing for Aim 2.
Funding Support:	NIH/NCI Brain Tumor SPORE; Multiple NIH/NCI R01s

Name:	Linda Liau, M.D., Ph.D.
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-4053-0052
Nearest Person Month Worked:	0.5
Contribution to the Project:	Dr. Liau is responsible for patient recruitment and obtaining image-guided biopsies for Aim 2.
Funding Support:	NIH/NCI Brain Tumor SPORE; Multiple NIH/NCI R01s

Name:	Robert Prins
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-6282-6583
Nearest Person Month Worked:	0.5
Contribution to the Project:	Dr. Prins is responsible for immunology tissue processing for Aim 3.
Funding Support:	NIH/NCI Brain Tumor SPORE; Multiple NIH/NCI R01s

Name:	Harley Kornblum
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-3779-4540
Nearest Person Month Worked:	0.5
Contribution to the Project:	Dr. Kornblum is responsible for single cell and stem cell analyses for Aim 2.
Funding Support:	NIH/NCI Brain Tumor SPORE

Changes in the Active Other Support for the PIs or Senior/Key Personnel Since Last Reporting Period

Benjamin M. Ellingson, Ph.D.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01 CA270027-01: Role of decorin and diffusion MRI in anti-VEGF efficacy for recurrent glioblastoma:**
  This new project will establish the mechanistic links between decorin (DCN) expression, diffusion MRI, and anti- VEGF treatment efficacy by first (Aim 1) performing a deep exploration into the association between diffusion MR phenotypes and DCN expression in human GBM using image-guided biopsies and examining DCN protein and gene expression, as well as the relationship with genotypes using whole exome analysis, genetic subtypes using bulk RNA sequencing, cellular states using single-cell RNA sequencing, and blood plasma levels of circulating DCN. Concurrently, we will (Aim 2) establish the causal links between DCN expression, diffusion MRI measurements, and anti-VEGF treatment in GBM by conducting a genetically modified patient-derived orthotopic xenograft (PDX) preclinical trial through editing a series of patient-derived cell lines to silence of overexpress DCN within PDX models using a tetracycline-controlled gene expression system, then perform diffusion MRI and treat with anti-VEGF therapy.

- **NIH/NCI P50 CA211015-06: UCLA Brain Tumor SPORE - Core 2: Neuro-Imaging Core (NIC):**
  The primary goal of the Neuro-Imaging Core (NIC) is to provide advanced MRI and PET imaging support with established reliability and consistency to SPORE project investigators for their respective projects. Specifically, the NIC will use quantitative μMRI and μPET for pre- clinical imaging (Aim 1) and state-of-the-art MR and PET imaging acquisition, advanced post-processing, and novel analysis tools for clinical imaging of patients in novel clinical trials (Aim 2). Additionally, we will provide professional expertise and resources for traditional and enhanced radiographic response assessment for the clinical trials for individual SPORE projects (Aim 3). Hence, this Core will add value to the overall SPORE by helping investigators to better understand the in situ metabolic, physiologic, functional, and traditional radiographic changes within the brain and tumor to address specific objectives of each of the UCLA Brain Cancer SPORE projects. Another main goal of the NIC is to understand the link between metabolic and/or physiologic imaging and tumor biology to reliably delineate treatment response versus recurrent tumor, which in turn will facilitate the direct translation of new MR-PET companion biomarkers/techniques to the clinic for the evaluation of treatment response in ongoing and new therapeutic clinical trials resulting from the SPORE projects and cores.

David Nathanson, Ph.D.

- **NIH/NCI P50 CA211015-06: UCLA Brain Tumor SPORE – Project 2: Overcoming drug-induced resistance to intrinsic apoptosis in glioblastoma:** *This SPORE Project renewal will test this hypothesis through the following aims. Aim 1 will investigate whether a novel BCL-xL antagonist (in collaboration with Abbvie) with GBM specificity has anti-tumor effects when combined with TMZ/IR or a new, clinical brain penetrant EGFR TKI in preclinical GBM models. Aim 2 includes a "window of opportunity" clinical trial to explore whether these novel clinical drugs can ablate the two intrinsic apoptotic blocks in recurrent GBM patients. Finally, Aim 3 proposes to identify potential mechanisms of resistance to targeting the intrinsic apoptotic machinery in diverse preclinical GBM samples. Together, the studies proposed in this application present a new therapeutic paradigm through specific manipulation of intrinsic apoptotic pathways in malignant glioma and have the long-term potential to shift current approaches in glioma therapy.*

Linda Liau, M.D., Ph.D.

- **NIH/NCI P50 CA211015-06: UCLA Brain Tumor SPORE**
  Overall, the UCLA Brain Cancer SPORE will support research into new and innovative strategies to diagnose and treat brain cancer, particularly focusing on innovative ways to overcome the problem of treatment-induced resistance to current therapies (i.e., immunotherapy, chemo/drug therapy, and radiation therapy resistance). Despite many different treatment approaches, the five-year survival rate for glioblastoma (WHO grade IV) patients remains dismal, and there are still no definitive cures for this disease. Thus, the proposed research is of relevance to public health, as there is clearly an unmet need for patients with this type of cancer and continued investigations into novel therapeutic approaches for brain cancer are desperately needed.

Robert Prins, Ph.D.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01 CA2667726-01: Neoadjuvant checkpoint blockade for recurrent glioblastoma:**
  This new project will investigate how pre-surgical/neoadjuvant immunotherapy may alter the systemic immune response and modify the tumor immune microenvironment in recurrent glioblastoma patients. We will take advantage of our unique access to biological samples, clinical data, and imaging data from an ongoing investigator-initiated clinical trial with Bristol Myers Squibb testing PD-1 (nivolumab) with or without CTLA-4 (ipilimumab) antibody blockade in the neoadjuvant setting and following a planned surgical resection. This project could potentially be transformative, as a better understanding of how neoadjuvant immunotherapy alters immune responses within the tumor could teach us important lessons about the critical requirements for productive anti-tumor responses in glioblastoma and how adaptive resistance occurs in this disease.

- **NIH/NCI P50 CA211015-06: UCLA Brain Tumor SPORE – Project 1: Targeting immunotherapy-induced resistance with DC vaccination and PD-1/CSF-1R inhibition:** *This SPORE Project renewal will investigate mechanisms of immune evasion following active immunotherapy with brain cancer vaccines. We will test our hypothesis that clinically relevant anti-tumor immunity to glioblastoma (GBM) must have two cellular components: 1) significant infiltration of tumor-specific tumor-infiltrating lymphocytes (TIL); and 2) blockade of immunosuppressive tumor infiltrating myeloid cell (TIM) function within the tumor microenvironment. We believe that a combination of active vaccination (ATL-DC; to induce T-cell infiltration into tumors), immune checkpoint inhibition (to enhance T cell function), and CSF-1R inhibition (to block suppressive TIM function) may lead to improved outcomes for the treatment of glioblastoma.*

Harley Kornblum, M.D.

- **National Institutes of Health (NIH) and National Institute of Neurological Diseases and Stroke (NINDS) R01 NS121617-01A1: Radiation-induced vascular reprogramming in glioblastoma:**
  The goals of the current studies are to understand the process of vascular reprogramming (RIR) and to determine how it influences brain tumor biology. Our hypothesis is that vascular reprogrammed cells provide critical trophic support to the remaining tumor cells under the harsh conditions that occur following radiation. First, in Aim 1 we will determine whether therapeutically relevant doses of radiation promote vascular RIR. We will then use cell ablation strategies to validate our preliminary data indicating that radiation-induced reprogramming is important for the subsequent growth of the tumor following radiation treatment using both xenotransplantation and immunocompetent syngeneic mouse models. Next, we will explore the mechanisms by which radiation reprogrammed endothelial-like and pericyte-like cells promote the growth of the remaining tumor, determining what specific factors they elaborate, and whether these factors are responsible for tumor survival and growth following radiation. We will then test the hypothesis that radiation induces the formation of vascular-like cells through modification of chromatin accessibility via augmentation of histone acetylation through the P300 histone acetyltransferase, allowing for access of vascular-specifying transcription factors. Finally, we will use pharmacologic agents to therapeutically target the process of RIR through inhibition of the P300 HAT. These experiments can lead to a new understanding of mechanisms underlying resistance to radiation therapy and open the door to new treatments.

Other Organizations Involved as Partners

Organization Name: Siemens Healthineers
Location of Organization: Erlangen, Germany
Partner's Contribution to Project: In-Kind and Collaboration Support. Siemens has provided an onsite engineer (Xiaodong Zhong, Ph.D.) to help with this project and others.

Organization Name: NMR Laboratory, Neuromuscular Investigation Center, Institute of Myology
Location of Organization: Paris, France
Partner's Contribution to Project: Collaboration support. Dr. Alfredo Lopez has been collaborating with us and helping us troubleshoot issues with our pulse sequence source code.

8. Special Reporting Requirements

Nothing to report.

9. Appendices

Nothing to report.